REMARKS

Reconsideration of the above-identified application in view of the amendments above and the remarks following is respectfully requested.

Claims 1-50 are in this case. Claims 38-50 have been withdrawn from further consideration, as being drawn to a non-elected invention. Claims 1-37 have been rejected. Claims 6, 10 and 18 have now been cancelled. Claims 1-5, 7-9, 11-17 and 19-37 have now been amended.

Information Disclosure Statement

Applicant has noted the Examiner's statement that the listing of references in the specification is not a proper information disclosure statement. Applicant wishes to direct the Examiner's attention to the Information Disclosure Citation (PTO Form 1449) filed for the instant application, received by the USPTO on October 25, 1999, an additional Information Disclosure Statement (PTO Form 1449) including copies of the Information Disclosure Statement filed in U.S. Patent Application Nos: 09/260,037, 09/140,888, 09/046,475, now US Patent No. 6,153,187, and 08/922,170, now US Patent No. 5,968,822, of which this application claims priority, filed December 25, 2002; and the Supplemental Information Disclosure Statement filed for the instant application, received by the USPTO April 30, 2003, as evidenced by the Mail Room Stamp.

Specification

The Examiner has objected to the disclosure on the basis of incomplete citation of "Murray RK and Keeley FW..." on page 3, line 6-7. The citation has been corrected to include publisher, city and year of publication, according to the Examiner's recommendations.

Claims Objections

The Examiner has objected to claims 6, 18 and 21-37 because of certain informalities.

Claims 6, 10 and 18 have now been cancelled, rendering moot the Examiner's rejection thereof. Thus, amended claims 21-37 no longer relate to methods recited in any of amended claims 1-20.

Further, independent claim 21 and claims dependent therefrom have now been amended to recite: "A method of improving in vitro fertilization (IVF) embryo implantation, the method comprising contacting an embryo generated via IVF with an effective amount of mammalian heparanase...", as recommended by the Examiner. Independent claims 26 and 31, and claims dependent therefrom have also been similarly amended, the term: "...an embryo generated via IVF..." replacing the term "...the IVF embryo...".

Applicant believes to have thus overcome all claims objections.

35 U.S.C. § 112 First Paragraph Rejections

The Examiner has rejected claims 1-37 under 35 U.S.C. § 112, First Paragraph, as containing subject matter which was not described in the specification in such a way as to convey to one skilled in the art that the inventors, at the time that the application was filed, had possession of the claimed invention. The Examiner's rejections are respectfully traversed. Claims 6, 10 and 18 have been cancelled, rendering moot the Examiner's rejection thereof. Claims 1-5, 7-9, 11-17 and 19-37 have now been amended.

The Examiner states that while claims 1-37 are directed to all possible methods of improving embryo implantation by contacting an embryo or a receptive uterus with an effective amount of heparanase and implanting the embryo in a receptive uterus, the specification only provides a single representative method, of improving murine embryo implantation comprising contacting a murine embryo with "CHO-p65" heparanase, and implanting the embryo in a receptive uterus. The Examiner further states that the specification lacks a disclosure of any particular structure to function/activity relationship in the disclosed heparanase or of representative methods other than those for murine embryos.

In strong contrast to the Examiner's contention, Applicant wishes to point out that the methods for improving embryo implantation of the present invention

comprise contacting the embryo, or uterus, or both with an effective amount of heparanase, said heparanase being clearly defined in the instant specification:

> "...heparanase refers to an animal endoglycosidase hydrolyzing enzyme which is specific for heparin or heparan sulfate proteoglycan substrates, as opposed to the activity of bacterial enzymes...The heparanase can be natural...or recombinant...modified, precursor...or activated form as described in, for example, PCT/US98/17954 and PCT US99/09256..." (page 18, line 24 to page 19, line 4).

Thus, heparanase, as defined in the instant specification, is an art-recognized term, distinguished from other ECM degrading enzymes such as hyaluronidase, chondroitinase, chondro-4 or chondro-6 sulfatase, heparan sulfatase, iduronate sulfatases, N-acetylglucosamine-6-sulfatase, etc., by substrate specificity, specific cleavage site and characteristic proteoglycan cleavage products (see, for example, Goldschmidt O et al., PNAS USA 2002;99:31-6, and Okada Y. et al., J Biol Chem, 2002;277:42488-95). Further, the heparanase activity detected in a wide variety of normal and malignant human cells and tissues is associated with the expression of a single, unique heparanase gene having significant homology to unique heparanase gene nucleotide sequences in rodent, bovine and even avian species (Vlodavsky, I and Freidman, Y. J. Clin Invest 2001;108:341-347), indicating that one dominant heparanase is expressed in animal cells.

Applicant wishes to further point out that the heparanase used in Example 2, the "CHO-65 heparanase" is recombinant human heparanase expressed in Chinese Hamster Ovary cells (for details, see US Patent Application No. 09/071,618, which has been incorporated into the instant specification by reference), identical in amino acid sequence, substrate specificity, kinetic characteristics catalytic activity, and antigenicity to an active heparanase enzyme characteristic of human tissues, having high homology to heparanase of other mammalians. Thus it would be clear to one of ordinary skill in the art that treatment of embryos, or uteri with heparanase, according to the methods of the present invention, can be affected by any one of the animal

heparanases, native or recombinant, having similar functional and structural characteristics, including, for example, rat, mouse or chicken heparanase (see, for example, US Patent Application No. 09/930,218 to Goldschmidt et al.).

The abovementioned notwithstanding, and to further define the present invention, the Applicant has chosen to amend independent claims 1-5, 7-9, 11-17 and 19-37 to include the limitation: "mammalian heparanase" in place of the recitation of "heparanase". Thus, the methods of amended independent claims 1, 7, 13, 21, 26 and 31, and claims directly and indirectly depending therefrom, now teach the use of "...an effective amount of mammalian heparanase...", and as such describe the claimed invention in clear, concise and exact terms. Support for such an amendment is found throughout the instant specification (see, for example, page 18, line 26 to page 19, line 14; and U.S. Patent Nos. 6,242,238; 5,968,822; 6,153,187; 6,177,545; and 6,190,875, the contents of which have been incorporated into the instant specification by reference - page 13, lines 25-30).

Further, Applicant notes that according to the revised "Guidelines for the Examination of Patent Applications Under the 35 USC 112, paragraph 1, 'Written Description' Requirement", section IB ("New or Amended Claims"), the guidelines clearly state that "there is no *in haec verba* requirement", such that the wording of the new or amended claims does not need to be literally present in the specification. Instead, it is sufficient if the wording is "supported in the specification through express, implicit, or inherent disclosure".

Thus, from the parent applications and the present Application, it is clear that the genuses of mammalian heparanase are taught.

The Examiner has further stated that disclosure of improving implantation of murine embryos, as described in the instant specification, fails to sufficiently describe the claimed invention in full, clear, concise and exact terms. Regarding the relevance of methods for implantation of murine embryos to implantation methods in other mammalian species, and in humans in particular, Applicant wishes to point out that the mouse model, in use for investigation of fertility and techniques for intervention in Reproductive Medicine for decades, is widely considered indispensable and predictive. Mouse models are not only convenient for experimental manipulation,

but are considered specifically suited for investigation into embryo implantation and other IVF techniques:

> "There are huge differences in reproductive physiology between the mouse, human and cow...Interactions between the embryo and corpus luteum are similar in cows and humans, but mouse and human embryo implantations are closer."(Menezo, YJ, and Herubel F, Reprod Biomed Online, 2002; 4:170-175, abstract enclosed).

Indeed, mouse and rat models have been extensively validated for optimization of in-vitro conditions in assisted reproductive-technology derived embryos (see Bean, et al. Human Reprod. 2002;17:2362-67, and Brinster, et al., Biol Reprod 2003;69:412-20, abstracts enclosed); optimization of conditions for use of cryopreserved spermatozoa and primordial follicles in IVF (Lui, J et al., Biol Reprod 2001; 64:171-78; and Kawase Y, et al., Biol Reprod 2002;66:381-85, abstracts enclosed); optimization of techniques for cryopreservation of embryos (Pfaff R. et al., Biol Reprod 2000;63:1294-1302, abstract enclosed); study of adhesion formation following endoscopic surgery (Yesildaglar N et al., Human Reprod 1999;14:55-59, abstract enclosed) and in the study of placental dysfunction and abruptio placentae (Ejima, et al. Biol Reprod 2000;62:178-85, abstract enclosed), among others. Of particular historical importance is the mouse model of transplacental exposure to diethylstilbestrol (DES), crucial in understanding the pathology attributed to the use of DES in high risk pregnancies in humans (see, for example, Prahalada S et al., Biol Reprod 1988;38:935-43; and Newbold, RR, et al., Biol Reprod 1983;28:735-744, abstracts enclosed). Indeed, many mouse strains have been specifically adapted for use in reproductive research: Jackson Laboratory (Bar Harbor, MA) offers more than 50 strains of mice specifically bred for Reproductive Biology (see enclosed list).

In view of the amendments and objective evidence presented hereinabove, the Applicant believes to have overcome the 35, U.S.C 112, first paragraph, rejections.

35 U.S.C. § 103 (a) Rejections: Yagel et al., Nakajima et al. and Fuks

The Examiner has rejected claims 1-37, as being unpatentable over Yagel et al. (J. Cellular Physiol, Vol.136, pages 455-462, 1988), Nakajima et al. (Heparanases and Tumor Metastasis, J. Cell Biochemistry, Vol. 36, pages 157-167, 1988) and Fuks (U.S. Patent No. 5,362,641) on the basis of obviousness. The Examiner's rejections are respectfully traversed. Claims 6, 10 and 18 have been cancelled, rendering moot the Examiner's rejection thereof. Claims 1, 3, 7, 13, 21, 26 and 31 have now been amended.

The Examiner states that Yagel et al. teach that the invasive ability of a trophoblast cell line is related to collagenase activity, laminin production and heparan-sulfatase activity, that Nakajima et al. teach a heparin sulfate degradative activity of metastatic B16 cells correlating with their lung-colonizing potential, and that Fuks et al. teach the purification of heparanase and i the desirability of it's use in wound healing, ovulation and transplantation. Based on the abovementioned teachings, the Examiner then concludes that:

> "one of ordinary skill in the art...would have been motivated to use the heparanase containing formulation of Fuks et al. to treat either an embryo, a receptive uterus, or both prior to implantation of the embryo...thus improving embryo implantation in the uterus".

Contrary to the Examiner's contention, Applicant is of the strong conviction that the prior art references of Yagel et al., Nakajima et al. and Fuks et al. do not relate, in any sense, to the methods for use of heparanase for improving embryo implantation taught and claimed in the instant application, and as such cannot anticipate, render obvious nor provide motivation for one of ordinary skill in the art to use heparanase for improving embryo implantation.

The present invention is of methods of improving embryo implantation comprising contacting a receptive uterus and/or embryo with an effective amount of mammalian heparanase and implanting the embryo in the receptive uterus. The present invention is based on experimental results that unexpectedly showed, for the

first time, that transgenic mice overexpressing heparanase have consistently larger litters than their wild-type controls (see Table 1, page 25). This motivated the inventors to investigate the effect on implantation success of preimplantation exposure of embryos and/or uteri to heparanase. As described in detail in the instant specification (Tables 3 and 4) it was surprisingly observed that treatment of preimplantation blastocysts, or of the uterus with a formulation comprising mammalian heparanase improved implantation rates by at least 40% over untreated controls in a murine model of assisted reproductive technology (IVF).

In stark contrast, Yagel et al. describe an in vitro "amnion invasion" system for assessing the "invasive properties" of cultured human trophoblast cells lines and metastatic B16F10 and C10 cells, demonstrating that the cultured trophoblasts can cross the amnion-derived barrier (Table 1), that proteinase inhibitors and antiplasminogen antibodies can block the cultured trophoblast cell invasion (Fig 5 and Table 2), and that an activator of procollagenase (mersalyl) enhances cultured trophoblast cell (Figure 6 and Table 3). The authors also measured "significant levels of collagenase (Table 4) and laminin (Table 5) in the trophoblast conditioned medium". The authors conclude that "...trophoblasts share some of the molecular mechanisms of invasion with metastatic cells...Plasmin generation was found to be an important step...A direct role for collagenase is suggested...Trophoblast cells also showed the ability to produce ...laminin."

However, these conclusions are far from sound. The trophoblast cell lines used in Yagel's study are established from "first trimester human placentae derived from elective termination of pregnancy". These cells were further cultured for at least 1-2 weeks. Thus, the cells used to establish the cell lines were from embryos up to 3 months old, a stage of development far later than that characteristic of human trophoblasts at implantation (trophoblast implantation typically occurs following blastocyst adhesion, at the 7^{th} and 8^{th} days of gestation in humans). Little is known of the relevance of later trophoblast activity to the implantation process:

> "How far maternal cellular responses to invading trophoblasts seen at later stages of pregnancy can be traced back to the implantation period is an open question"

(Pijnenborg et al. , Reprod Biomed Online 2002;4 Suppl 3:14-17).

Further, the relevance of the seeming similarity between metastatic invasive properties and trophoblast implantation has often been questioned:

> "In contrast to the trophoblast of the rabbit blastocyst, none of the invasive tumor cell lines tested was able to adhere to intact epithelium of endometrial fragments nor to penetrate it". [Hohn HP et al; Cells Tissues Organs 2003, 173(4):204-16].

> "In contrast to tumor invasion of a host tissue, trophoblastic invasion during implantation and placentation is stringently controlled both in space and time. The factors responsible for these important regulatory processes are unknown, but in-vitro studies point to autocrine (trophoblastic) and paracrine (endometrial) controls by cytokines and growth factors". (Bischof P, Campana A; Hum. Reprod. 2000, 15 Suppl 6:51-8).

> "Insulin-like growth factor binding protein 1, the major secretory product of the decidua, interleukin 1, interleukin 6, leptin and tumour necrosis factor alpha, all of endometrial origin, are stimulators of MMPs, whereas transforming growth factor beta inhibits the proteolytic activity of cytotrophoblastic cells. Unfortunately, the ways in which these individual factors interact to regulate trophoblast invasion are far from being understood". (Bischof P et al; J. Reprod. Fertil. Suppl. 2000, 55:65-71).
> "Although cytotrophoblastic cells behave like metastatic cells, in vivo they are only transiently invasive (first

> trimester) and their invasion is normally limited only to the endometrium and to the proximal third of the myometrium. This temporal and spatial regulation of trophoblast invasion is believed to be mediated in an autocrine way by trophoblastic factors and in a paracrine way by uterine factors. Several types of regulators have been investigated: hormones, cytokines, growth factors and ECM glycoproteins". (Bischof P et al; Placenta. 2000, 21 Suppl A:S55-60).

Further, and of greater significance, the Examiner has mistakenly quoted Yagel et al., equating their citation of "heparan sulfatase activity" (page 460, left column) with heparanase activity. Applicant wishes to point out that heparan sulfatase and heparanase are two distinct classes of enzyme activities, and the profound differences in substrate specificity, catalytic properties, and polypeptide structure of the proteins characteristic of each class are well known in the art.

Firstly, heparanase is an endoglycosidase:

> "Heparanase cleaves the glycosidic bond with a hydrolase mechanism, and is thus distinct from bacterial heparinases, which depolymerize heparin and HS by eliminative cleavage. HS glycosaminoglycan chains are cleaved by heparanase at only a few sites, yielding HS fragments of appreciable size (10-20 sugar units)...". (Vlodavsky and Friedmann, J Clin Invest, 2001, 108:341-347).

In sharp contrast, heparan sulfatase belongs to a group of lysosomal enzymes having exoglycosidic activity:

> "The major class of sulfatases are lysosomal enzymes, which are involved in the degradation of sulfated substrates in an acidic environment. These enzymes are exosulfatases, in that

> their activity is confined to sulfate esters on the non-reducing termini of saccharide units...One of the lysosomal sulfatases is G6S, acting on glucosamine 6-sulfate and GlcNAc-6-sulfate in the degradation of HSPGs... (Morimoto-Tomita et al., J Biol Chem 2002;277:491275-85).

> "The heparin/heparan sulfate 2-O-sulfatase exclusively hydrolyzes the sulfate at the 2-O-position of the uronic acid, which also exhibits...a clear kinetic preference for disaccharides". (Myette JR et al; J. Biol. Chem. 2003, 278(14):12157-66).

> "Sulfatases are responsible for the degradation of HS in the lysosome. Their substrate are mono-tri-saccharides". (Freeman C, Hopwood JJ; Biochem. J. 1987, 246(2):355-65).

Similarly, the heparanase and heparan sulfatase enzymes differ greatly in structure and molecular properties:

> "The (human) heparanase cDNA contains an open reading frame of 1629 bp encoding a 61.2 kDa polypeptide of 543 amino acids. The natural active 50 kDa enzyme ...has it's N-terminus 157 amino acids downstream from the initiation codon, suggesting post-translational processing...The active enzyme has been postulated to be a dimer." (Vlodavsky and Friedmann, J Clin Invest, 2001, 108:341-347).

Heparan sulfatase, on the other hand, is a monomer of considerably greater molecular mass, having :

> "N-acetylglucosamine-6-sulfatase cleaves 6-sulfate esters present in non-reducing terminal glucosamine-6-sulfate, 2-

sulfamino glucosamine-6-sulfate or N-actylglucosamine-6-sulfate. The enzyme is also active towards monosaccharide substrates. The enzyme is an exosulfatase since it is unable to cleave internal 6-sulfate esters. Unlike heparanase, the molecular weight of the recombinant enzyme is reported to be 102 kD by gel filtration and 94 kD by SDS-PAGE. Unlike heparanase, enzyme activity is apparently stimulated by the presence of bovine serum albumin in reaction mixtures, most probably due to a stabilizing effect on the enzyme during incubations performed at elevated temperatures. Sulfate and phosphate ions are potent inhibitors of activity".(www.informatics.jax.org/searches).

"Iduronate-2-sulfatase is an exosulfatase which removes 2-sulfate esters from non reducing terminal iduronate 2-sulfate of heparin/heparan sulfate and dermatan sulfate. The recombinant enzyme is glycosylated and exhibits a broad band at 85-90 kD by SDS-PAGE. Unlike heparanase, enzyme activity is apparently stimulated by the presence of 0.1 mg/ml bovine serum albumin in reaction mixtures, most probably due to a stabilizing effect on the enzyme during incubations performed at elevated temperatures. Enzyme activity is inhibited by NaCl. Phosphate and sulfate ions are potent inhibitors of activity (www.informatics.jax.org/searches/).

Indeed, a search of the NCBI BLAST protein database using the term "human heparanase" reveals none of the proteins identified using the term "human heparan sulfatase" (see enclosed search results).

Further, the significance of these differences in substrate specificity and catalytic activity can be appreciated when observing the difference in biological effects of heparanase and heparan sulfatase activity, for example: Heparanase

degradation of HS activates the important cytokine basic FGF, while the desulfated HS degradation products produced by heparan sulfatase fail to release ECM-bound basic FGF (Ishai-Michaeli R et al. Biochem 1992;31:2080-88).

Careful reading of Yagel et al. reveals that a single reference is made to heparan sulfatase activity, while no mention is made of the importance of heparanase or heparanase activity. Thus, in view of the evidence brought hereinabove, Yagel et al. clearly do not teach that, "in addition to collagenase, heparanase is an important activity related to invasive ability", as stated by the Examiner, but rather teach away from the consideration of the role of heparanase. As such, Yagel et al. provide no motivation for the use of heparanase to treat either an embryo, a receptive uterus or both as claimed.

Regarding Fuks et al., the Examiner states that Fuks et al. teach the purification of heparanase and its use in formulations for therapies in which the release of FGF is desirable, such as wound healing, ovulation and transplantation. Thus, the Examiner concludes that in light of the teachings of Fuks et al., and Yagel et al., one of ordinary skill in the art would have been motivated to use the heparanase containing formulation to treat either an embryo, a receptive uterus or both prior to embryo implantation, as a means of improving embryo implantation.

Applicant wishes to point out that, contrary to the Examiner's contention, Fuks et al. do not teach, nor do they imply, the use of heparanase for improving embryo implantation. Fuks et al. teach that heparanase "appears to play an integral role in a number of specific physiological functions, such as tumor metastasis and autoimmune disorders", and that "heparanase also has the effect of causing release of angiogenic endothelial cell growth factors (such as FGF) from basement membranes and subendothelial ECM". Fuks et al. further describe the partial purification of heparanase from sk-Hep-1 cells (Fuks' preparation of heparanase remained contaminated by PAI-1 protein), and teach the use of heparanase in formulations "for the treatment of wounds, and enhancement of the wound-healing process...also for the treatment of any other physiological state or condition in which neovascularization or angiogenesis would be expected to be of benefit", "cardiac, cerebral and peripheral ischaemic diseases and diseases associated with vascular damage, such as diabetes, hypertension and systemic lupus

erythematosus...ovulation, hair growth, transplantation, nerve regeneration and bone and cartilage repair". No mention of fertilization, trophoblasts, gestational processes or embryonic development is made. The terms "ovulation" and "transplantation" are clearly not related to embryo implantation, neither in the context of the cited prior art document, nor according to art-recognized definitions:

> "Placentation: The development of the placenta and attachment of the fetus to the uterus during pregnancy."
>
> "Transplantation: The removal of tissue(s) from one part of the body, or from one individual, and its implantation, or insertion in another, especially by surgery."
>
> "Implantation: A) The placement of a natural or artificial tooth in an artificially prepared socket in the jawbone. B) The process of attachment of the embryo to the maternal uterine wall called also nidation. C) Medical treatment by the insertion of an implant."
>
> "Ovulation: The discharge of a mature ovum from the ovary" (Merriam-Webster)

Further, in the examples taught by Fuks et al., the action of heparanase on ECM-bound factors is demonstrated, the purification of heparanase from sk-hep-1 cells is demonstrated, and the detection of heparanase activity in ovarian tumor tissue is demonstrated. No examples of therapeutic use of heparanase are described or exemplified.

Thus, it is the Applicant's strong opinion that Fuks et al. alone, or in combination with the teachings of Yagel et al., do not teach, nor provide motivation for treatment of receptive uteri, embryos, or both with heparanase formulations to improve embryo implantation.

Regarding the prior art document of Nakajima et al., the Examiner has stated that Nakajima et al. teach that heparan sulfate degradative activity is associated with invasive metastatic cells and normal placenta, and as such one of ordinary skill in the art would have been motivated to use the heparanase for treatment of receptive uteri, embryos, or both with heparanase formulations to improve embryo implantation.

Applicant wishes to point out that the heparanase taught by Nakajima et al. in 1988 has since been identified as distinct from mammalian heparanase, originating in contamination of the enzyme preparation. The mammalian heparanase recited in context of the present invention is the heparanase enzyme purified, isolated and cloned by Vlodavsky et al. (see, for example, U.S. Patent Nos. 6,242,238; 5,968,822; 6,153,187; 6,177,545; and 6,190,875, the contents of which have been incorporated into the instant specification by reference) having a molecular mass of ca. 50 kDa, in the mature dimer form, processed from a 65 kDa pre-proheparanase polypeptide. In stark contrast, the enzymatic activity isolated from B16 melanoma cells, taught by Nakajima et al., in the cited prior art document from 1988, is a polypeptide having molecular mass of about 96 kDa, with endo-β-D-glucoronidase activity having endo-β-D-glucoronosyl-N-acetylglucosaminyl linkage specificity. The amino terminal sequence of the melanoma heparanase taught by Nakajima et al. was later found to be characteristic of a 94 kDa glucose-regulated protein (GRP94/endoplasmin) lacking heparanase activity (Mollinedo, F., et al. Biochem. J., 1997; 327:917-923), suggesting that the endoplasmin-like 96 kDa protein reported by Nakajima et al. in melanoma heparanase preparations is a contaminant (Mollinedo, F., et al. Biochem. J., 1997; 327:917-923, De Vouge, M.W., et al. Int. J. Cancer 1994, 56: 286-294).

In view of the evidence cited hereinabove, it is clear that one of ordinary skill in the art, in possession of the teachings of Fuks et al., Yagel et al. and/or Nakajima et al. would have had no motivation to use a heparanase formulation to treat either an embryo, a receptive uterus, or both prior to implantation, for improving implantation in the uterus. Thus, the present invention is not made obvious, nor is it anticipated by Yagel, et al., Fuks et al., and/or Nakajima et al., taken alone or in any combination.

In view of the above amendments and remarks it is respectfully submitted that now amended independent claims 1, 7, 13, 21, 26 and 31 and claims 2-5, 8, 9, 11, 12, 14-17, 19, 20, 22-25, 27-30 and 32-37 directly or indirectly depending therefrom are now in condition for allowance. Prompt notice of allowance is respectfully and earnestly solicited.

Respectfully submitted,

Sol Sheinbein
Registration No. 25,457

Date: July 31, 2003.

Enclosed:

One month extension fee.
Supplemental Information Disclosure Statement received by the USPTO April 30, 2003.
Information Disclosure Citation (PTO Form 1449) received by the USPTO October 25, 1999.
Information Disclosure Statement (PTO Form 1449) including copies of the Information Disclosure Statement filed in U.S. Patent Application Nos: 09/260,037, 09/140,888, 09/046,475, now US Patent No. 6,153,187, and 08/922,170, now US Patent No. 5,968,822, filed December 25, 2002.

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Menezo, YJ, and Herubel F, Reprod Biomed Online, 2002; 4:170-175
Bean, et al. Human Reprod. 2002;17:2362-67
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Prahalada S et al., Biol Reprod 1988;38:935-43
Newbold, RR, et al., Biol Reprod 1983;28:735-744
Reproductive Biology Mouse strains: Jackson Laboratory (Bar Harbor, MA)
NCBI Blast protein search

APPLICANT	:	Oron YACOBY-ZEEVI
DOCKET NO.	:	01/22716
SERIAL NO.	:	09/978,297
FILED	:	October 17, 2001
FOR	:	... Improving Implantation of Embryos

Receipt of the following papers is acknowledged by the U.S. Patent & Trademark Office as evidenced by the Mail Room Stamp:

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Applicant:	§	
Oron YACOBY-ZEEVI	§	
Serial No.: 09/978,297	§	
Filed: October 17, 2001	§	Group Art Unit: 1633
For: Methods of and Pharmaceutical Compositions for ...	§	Attorney Docket: **01/22716**
Examiner:	§	

Commissioner of Patents and Trademarks
Washington, DC 20231

INFORMATION DISCLOSURE STATEMENT

Sir:

Enclosed is a PTO Form 1449 which lists citations which may be material to the patentability and examination of the above identified application. Also enclosed are copies of the references cited thereon. These are submitted in compliance with the duty of disclosure defined in 37 CFR 1.56. The Examiner is requested to make these citations of official record in this application.

This application is a Continuation of U.S. Patent Application No. 09/260,037, filed March 2, 1999, which is a Continuation-In-Part of U.S. Application Patent No. 09/140,888, filed August 27, 1998, which is a Continuation-In-Part of U.S. Application Patent No. 09/046,475, filed March 25, 1998, now U.S. Patent No. 6,153,187, issued November 28, 2000, which is a Continuation-In-Part of U.S. Application Patent No. 08/922,170, filed September 2, 1997, now U.S. Patent No. 5,968,822, issued October 19, 1999.

Enclosed herewith is: a copy of the Information Disclosure Statement filed in U.S. Patent Application No. 09/260,037, filed March 2, 1999; copies of the Information Disclosure Statement and Notice of References Cited filed in U.S. Application Patent No. 09/140,888, filed August 27, 1998 and a copy of the resultant patent, U.S. Patent 6,423,312 issued July 23, 2002; copies of the Information Disclosure Statement and Notice of References Cited filed in U.S. Patent Application No. 09/046,475 as well a a copy of the resultant patent, U.S. Patent No. 6,153,187 issued November 28, 2000; a copy

of the Information Disclosure Statement filed in U.S. Patent Application No. 08/922,170 as well as a copy of the resultant patent, U.S. Patent No. 5,968,822. Copies of the art cited are not submitted herewith.

Applicant requests that the art cited in the above prior applications be considered in this application and be cited in any resultant patent.

This Information Disclosure Statement under 37 CFR 1.56 is not to be construed as a representation that a search has been made, that additional matter which is material to the examination of this application does not exist, or that any or more of these citations constitutes prior art.

Respectfully submitted,

Sol Sheinbein
Attorney for Applicant
Registration No. 25,457

December 25, 2002

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PTO/SB/08A

(10-96)

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Substitute for form 1449A/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(use as many sheets as necessary)

Complete if Known	
Application Number	09/978,297
Filing Date	10/17/2001
First Named Inventor	Yacobi-Zeevi
Group Art Unit	1633
Examiner Name	
Attorney Docket Number	01/22716

Sheet	1	Of	4

U.S. PATENT DOCUMENTS

Examiners Initials	Cite No.[1]	U.S. Patent Document Number	Kind Code[2] (if known)	Name of Patentee or Applicant of Cited Document	Date of Publication of Cited Document MM-DD-YYYY	Pages, columns, lines, Where Relevant Passages or Relevant Figures Appear
	AA	5,688,679		Powell	11-18-1997	

FOREIGN PATENT DOCUMENTS

Examiners Initials	Cite No.[1]	Foreign Patent Documents Office[3]	Number[4]	Kind Code[5] (if known)	Name of Patentee or Applicant of Cited Document	Date of Publication of Cited Document MM-DD-YYYY	Pages, columns, lines, Where Relevant Passages or Relevant Figures Appear	T[6]
	AB	PCT	99/21975		Australian Nat'l University	05-06-1999		

Examiner Signature		Date Considered	

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

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Complete if Known	
Application Number	09/978,297
Filing Date	10/17/2001
First Named Inventor	Yacobi-Zeevi
Group Art Unit	1633
Examiner Name	
Attorney Docket Number	01/22716

Sheet 2 Of 4

Examiner Initials	Cite No.[1]	OTHER PRIOR ART – NON PATENT LITERATURE DOCUMENTS	T[2]
	BA	"The Merck Manual", R. Berkow, M.D. Ed-in-Chief, Merck Research Laboratories, 1997, pp 201, 204, 1308, 177-179, 1016-1017, 194-197, 885, 601.	
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	BH	Vlodavsky et al, "Expression Heparanase by Platelets and Circulating Cells of the Immune System: Possible Involvement in Diapedesis and Extravasation", *Invasion Metastasis*, 1992; 12(2):112-127 (Abstract)	
	BI	Naparstek et al, "Activated T Lymphocytes Produce a Matrix-Degrading Heparan Sulphate Endoglycosidase", *Nature*, 1984 July 19-25; 310(5974):241-244 (Abstract)	
	BJ	Armstrong et al, "Lower Airway7 Inflamation in Infants and Young Children with Cystic Fibrosis', *Am J Respir Crit Care Med*, 1997 Oct; 156(4Pt 1):1197-1204 (Abstract)	
	BK	Tang et al, "Contribution of Specific Pseudomonas Aeruginosa Virulence Factors to Pathogenesis of Pneumonia in a Neonatal Mouse Model of Infection", *Infect Immun*, 1996 Jan; 64(1):37-43 (Abstract)	
	BL	Murray et al, "The Extracellular Matrix", found in Harper's Biochemistry, 24th Ed., McGraw-Hill Professional 1998, Chap. 57, pp 667-679	
	BM	Selvan et al, "Heparan Sulfate in Immune Responses", *Annals New York Academy of Sciences*, 797:127-139, 1996	
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Filing Date	10/17/2001
First Named Inventor	Yacobi-Zeevi
Group Art Unit	1633
Examiner Name	
Attorney Docket Number	01/22716

Sheet 3 Of 4

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	CB	Rubin, Bruce K., "Emerging Therapies for Cystic Fibrosis Lung Disease", *Chest*, 115:1120-1126, 1999	
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✓	CE	Marty et al, "Influence of Nutrient Media on the Chemical Composition of Exopolysaccharide from Mucoid and Non-Mucoid Pseudom Aeruginosa", *FEMS Microbiol Lett*, 1992 Nov 1; 77(1-3):35-44 (Abstract)	
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✓	CL	Welch et al, "Complex Saccharide Metabolism In Cystic Fibrosis Fibroblasts" *Pediatr Res.* 1975 Sep;9(9):698-702. (Abstract)	
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✓	CN	Beuth et al, "Lectin-Mediated Bacterial Adhesion To Human Tissue", *Eur J Clin Microbiol*, 1987 Oct;6(5):591-3. (Abstract)	
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✓	CP	Albus et al, "Staphylococcus Aureus Capsular Types And Antibody Response To Lung Infection In Patients With Cystic Fibrosis", *J Clin Microbiol.* 1988 Dec; 26(12):2505-9. (Abstract)	
✓	CQ	Macone et al, "Mucoid Escherichia Coli In Cystic Fibrosis", *N Engl J Med.* 1981 Jun 11;304(24):1445-9. (Abstract)	

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Group Art Unit	1633
Examiner Name	
Attorney Docket Number	01/22716

Sheet 3 Of 4

✓	DA	Golberg et al, "An Improved Method For Determining Proteoglycans Synthesized by Chondrocytes in Culture", *Connective Tissue Research*, 24:265-275, 1990	
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	DJ		

Examiner Signature		Date Considered	

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PTO-1449 (Modified)

Atty. Docket No.	Application No. 09/260,037

INFORMATION DISCLOSURE CITATION IN AN APPLICATION
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Applicant: Oron YACOBY-ZEEVI et al	RECEIVED OCT 25 1999
Filing Date: March 2, 1999	Group Art Unit: 1643 TECH CENTER 1600/29

U.S. PATENT DOCUMENTS

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FOREIGN PATENT DOCUMENTS

		DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUB-CLASS	TRANSLATION	
							YES	NO
AB								

OTHER ART (Including Author, Title, Date, Pertinent Pages, Etc.)

AC	R.H.	Murry et al, "The Extracellular Matrix", found in "Biochemistry", Chap. 57, pp 667-685
AD	R.H.	Selvan et al, "Heparan Sulfate in Immune Responses", *Ann. NY Acad. Sci.*, 797: 127-139, 1996
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AJ	RH	Vlodavsky et al, "Extracellular Matrix-Bound Growth Factors, Enzymes, and Plasma Proteins", *Cell. Molec. Aspects*, 1993, Academic Press, Inc. Pp 327-343
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AL	RH.	Prockop, DJ, "Marrow Stromal Cells as Stem Cells for Nonhematopoietic Tissues", *Science*, 276: 71-74, 1997Krivit et al, "Microglia: The Effector cell for reconstitution of the Central Nervous System Following Bone Marrow Transplantation for Lysosomal and Peroxisomal Storage Diseases", *Cell Transplant*, 4(4): 385-392, 1995 (Abstract)
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EXAMINER	Richard Hutz	DATE CONSIDERED	12/22/99

PTO-1449 (Modified)

INFORMATION DISCLOSURE CITATION IN AN APPLICATION
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Atty. Docket No.	Application No. 09/260,037
Applicant: Oron YACOBY-ZEEVI et al	
Filing Date: March 2, 1999	Group Art Unit: 1643

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BB								

OTHER ART (Including Author, Title, Date, Pertinent Pages, Etc.)

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BD		Benathan et al, "Living Epidermal and Dermal Substitutes for Treatment of Severely Burned Patients", *Rev Med Suisse Romande*, 118(2): 149-153, 1998 (Abstract- art in French)
BE	RH	Wang et al, Basic Fibroblast Growth Factorn Enhances Bone-Graft Incorporation: Dose and Time Dependence in Rats", *J. Orthop Res*, 14(2): 316-23, 1996 (abstract)
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BG	RH	Garner WL, "Epidermal Regulation of Dermal Fibroblast Activity", *Plast Reconstr Surg*, 102(1): 135-139, 1998 (abstract)
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BO		

EXAMINER Richard Whitson DATE CONSIDERED 12/22/99

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INFORMATION DISCLOSURE CITATION IN AN APPLICATION
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09/260,037

Applicant: Oron YACOBY-ZEEVI et al

Filing Date: March 2, 1999

Group Art Unit: 1643

U.S. PATENT DOCUMENTS

	EXAMINER INITIAL	DOCUMENT NUMBER	DATE	NAME	CLASS	SUB-CLASS	FILING DATE
CA							

FOREIGN PATENT DOCUMENTS

		DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUB-CLASS	TRANSLATION	
							YES	NO
CB								

OTHER ART (Including Author, Title, Date, Pertinent Pages, Etc.)

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EXAMINER Richard Schwartzman DATE CONSIDERED 12/22/99

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PTO-1449 (Modified)

Atty. Docket No.	Application No. 09/260,030
Applicant: Oron YACOBY-ZEEVI et al	
Filing Date: March 2, 1999	Group Art Unit: 1643

INFORMATION DISCLOSURE CITATION IN AN APPLICATION
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DB								

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Notice of References Cited

Application No.	Applicant(s)		
09/260,037	Yacoby-Zeevi		
Examiner		Group Art Unit	Page 1 of 1
Richard Hutson		1652	

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	DOCUMENT NO.	DATE	NAME	CLASS	SUBCLASS
A	5,362,641	11/1994	Fuks et al.	435	209
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Volume 4, No 2 March/April 2002

Mouse and bovine models for human IVF

Yves Ménézo, François Herubel
Laboratoire Marcel Merieux, 1 Rue Laborde, 69500 Bron, France

It is obvious that the first prerequisite is to define for what purpose a mc needed for humans. There are huge differences in reproductive phys between the mouse, human and cow. As far as maturation is concerne plasticity of the mouse model is not the same in cows and humans. Th stages of oocyte maturation seem to be more finely regulated in cow humans, where a minimum size of follicle is necessary to complete matura vitro. Bovine and human preimplantation embryos seem to be more sim term of bochemical and intrinsic paternal and maternal regulatory proc Once again, interactions between the embryo and the corpus luteum are in cows and humans, but mouse and human embryo implantations are Mouse oocytes and embryos should not be overlooked, but exc generalization between mammalian species must be avoided.

Paper based on contribution presented at the Alpha meeting in New York, September 2001.

Reproductive BioMedicine Online 2002 Vol. 4, No. 2 170-175

Keywords: bovine, embryos, human, implantation, mouse, oocytes

Webpaper 2001/248

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HUMAN REPRODUCTION

Human Reproduction, Vol. 17, No. 9, 2362-2367, September 2002

Fertilization *in vitro* increases non-disjunction during early cleavage divisions in a mouse model system

Christopher J. Bean, Terry J. Hassold, LuAnn Judis and Patricia A. Hunt[1]

Department of Genetics and the Center for Human Genetics, Case Western Reserve University and University Hospitals of Cleveland, Cleveland, OH, USA

BACKGROUND: We have been studying an unusual mouse the BALB/cWt (Wt) male in which the Y chromosome is susceptible to high rates of mitotic non-disjunction, particularly at the first two cleavage divisions. As these are the same divisions that human embryos generated through assisted reproductive technology must complete in an artificial setting, analysis of the Wt Y chromosome allows us to examine the effect of fertilization and culture *in vitro* on mammalian chromosome segregation. METHODS: We performed standard mouse IVF, cultured embryos in 5% CO_2 in air or in a lowered oxygen atmosphere, and used fluorescence in-situ hybridization to examine the sex chromosome constitutions of 2-, 4-, 8- and 16-cell stage Wt Y-bearing embryos. RESULTS: We observed a significant increase in mosaic sex chromosome aneuploidy at each embryonic stage in embryos cultured in 5% CO_2 in air, but under lowered oxygen conditions mosaicism returned to control (in-vivo) levels. CONCLUSIONS: Our results demonstrate that slight alterations in in-vitro conditions may have a considerable impact on the genetic quality of assisted reproductive technology-derived embryos and suggest that the genetic quality of embryos should be a fundamental concern in the development of new culture systems for clinical use.

Keywords: fertilization in-vitro • non-disjunction • preimplantation embryo • Y chromosome

[1] To whom correspondence should be addressed at: Department of Genetics, Case Western Reserve University, 10900 Euclid Avenue, Cleveland, OH 44106, USA. E-mail: pah13@po.cwru.edu

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BIOLOGY OF REPRODUCTION 69, 412–420 (2003)
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Testis

Restoration of Fertility by Germ Cell Transplantation Requires Effective Recipient Preparation[1]

Clayton J. Brinster , Buom-Yong Ryu , Mary R. Avarbock , Levent Karagenc , Ralph L. Brinster and **Kyle E. Orwig** [2]

Department of Animal Biology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104

Spermatogonial transplantation provides access to the mammalian germline and has been used in experimental animal models to study stem cell/niche biology and germline development, to restore fertility, and to produce transgenic models. The potential to manipulate and/or transplant the germline has numerous practical applications that transcend species boundaries. To make the transplantation technology more broadly accessible, it is necessary to develop practical recipient preparation protocols. In the current study, mouse recipients for spermatogonial transplantation were prepared by treating pregnant females with the chemotherapeutic agent busulfan at different times during gestation. Donor germ cells were introduced into the testes of male progeny between 5 and 12 days postpartum. Analysis of recipient animals revealed that busulfan treatment of pregnant females on 12.5 days postcoitum was the most effective; male progeny transplanted with donor germ cells became fertile and passed the donor genotype to 25% of progeny. This approach was effective because 1) the cytoablative treatment reduced (but did not abolish) endogenous spermatogenesis, creating space for colonization by donor stem cells, 2) residual endogenous germ cells contributed to a healthy testicular environment that supported robust donor and recipient spermatogenesis, and 3) fetal busulfan-treated males could be transplanted as pups, which have been established as better recipients than adults. Laboratory mice provide a valuable experimental model for developing the technology that now can be applied and evaluated in other species.

[1] Histological sections were produced in the University of Pennsylvania Institute for Human Gene Therapy Morphology Core (grant 5-P30-DK-47747-07). Financial support for the research was from the National Institutes of Child Health and Human Development Grant 36504, the Commonwealth and General Assembly of Pennsylvania, and the Robert J. Kleberg, Jr. and Helen C. Kleberg Foundation.

[2] Correspondence: K.E. Orwig, Department of Animal Biology, School of Veterinary Medicine, University of Pennsylvania, 3850 Baltimore Ave., Philadelphia, PA 19104. FAX: 215 898 0667; korwig@vet.upenn.edu

Biology of Reproduction **64**, 171-178 (2001)

Regular Article

Live Offspring by In Vitro Fertilization of Oocytes from Cryopreserved Primordial Mouse Follicles after Sequential In Vivo Transplantation and In Vitro Maturation[1]

Jun Liu [2,,a], **Josiane Van der Elst** [a], **Rudy Van den Broecke** [b] and **Marc Dhont** [a,b]

[a] *Infertility Center, Department of Obstetrics and Gynecology and* [b] *Department of Gynecologic Oncology, Ghent University Hospital, B-9000 Ghent, Belgium*

ABSTRACT

The objectives of the present study were to achieve 1) oocyte maturation, 2) oocyte competence of fertilization, and 3) oocyte competence of embryogenesis with oocytes from primordial follicles obtained from cryopreserved newborn mouse ovaries by using a two-step method. In the first step, frozen-thawed newborn mouse ovaries were transplanted under the kidney capsule of recipients for the initiation of growth from the primordial follicle stage on. In the second step, growing preantral follicles in the ovarian grafts were recovered and cultured. The results demonstrated that primordial follicles were able to be recruited to preantral follicles during the period of transplantation, and preantral follicles could be mechanically isolated from ovarian grafts. Under the present in vitro culture conditions, 85.8% of the isolated follicles (n = 332) from ovarian grafts survived the 12-day in vitro culture process, 84.9% of the recovered oocytes (n = 285) were germinal vesicle breakdown (GVBD)-competent, and 76% of the oocytes that underwent GVBD (n = 242) developed to the metaphase II (MII) stage. In the in vitro fertilization experiments, 75.4% of 142 inseminated MII oocytes underwent fertilization and cleavage to the 2-cell stage. Subsequently, 79.7% of the 2-cell-stage embryos (n = 69) progressed to the late morula-early blastocyst stage. Transfer of late morula-early blastocyst embryos resulted in the production of live offspring. From our experiments, it may be concluded that in vivo maturation by grafting followed by in vitro maturation of frozen-thawed primordial follicles can restore fertility in mice. This model could be useful for a similar application in the human.

FOOTNOTES

First decision: 7 July 2000.

[1] Supported by a research grant from the Bijzonder Onderzocksfonds of Ghent University, Belgium (grant BOF01112199).

[2] Correspondence: Jun Liu, Infertility Center, Ghent University Hospital, De Pintelaan 185, B-9000 Ghent, Belgium. FAX: 32 9 240 4972; jun.liu@rug.ac.be

This article has been cited by other articles:

Biology of Reproduction 66, 381-385 (2002)

Regular Article

Effect of Partial Incision of the Zona Pellucida by Piezo-Micromanipulator for In Vitro Fertilization Using Frozen-Thawed Mouse Spermatozoa on the Developmental Rate of Embryos Transferred at the 2-Cell Stage

Yosuke Kawase [c,d], **Takamitsu Iwata** [c], **Otoya Ueda** [c], **Nobuo Kamada** [c], **Takanori Tachibe** [d], **Yukari Aoki** [d], **Kou-ichi Jishage** [c] and **Hiroshi Suzuki** [1,,2,,c]

[c] *Chugai Pharmaceutical Co., Ltd. and* [d] *CSK Research Park, Inc., Gotemba, Shizuoka 412-8513, Japan*

Cryopreservation of mouse spermatozoa is widely used, although considerable strain differences in fertilization rates using frozen-thawed mouse spermatozoa have been described. The C57BL/6 mouse strain is a very widely used for establishment of transgenic mice, but the fertilization rate associated with the use of cryopreserved C57BL/6 spermatozoa is very low compared with rates for other inbred strains. We have recently solved this difficulty by in vitro fertilization (IVF) in combination with partial zona pellucida dissection (PZD). However, this technique requires culture of fertilized eggs with PZD in vitro up to morula or blastocyst stage before transfer into the uterus because blastomeres are lost after transfer into the oviduct because of the relatively large artificial slit in the zona pellucida. To overcome this problem, we performed a partial zona pellucida incision by using a piezo-micromanipulator (ZIP) for IVF with frozen-thawed mouse spermatozoa. The blunt end of the micropipette touched the surface of the zona pellucida of the oocytes, and piezo pulses were used to incise the zona pellucida while the pipette was moved along by the surface of zona pellucida. The length of the incision was $\pi r/6$ μm. When cumulus-free ZIP and PZD oocytes were inseminated with frozen-thawed genetically modified C57BL/6J spermatozoa, the fertilization rates of ZIP and PZD oocytes were 52% and 48%, respectively. After embryo transfer at the 2-cell stage, 18% and 2% of the transferred embryos with ZIP and PZD developed to term, respectively. This difference was significant ($p < 0.05$). When ZIP and PZD zygotes were cultured to blastocyst stage and subsequently transferred to uterine horns of recipient animals, the difference between ZIP and PZD zygotes for development rate to full term was not significant. Our results indicate that ZIP is an effective alternative technique for IVF using cryopreserved mouse spermatozoa and subsequent embryo transfer.

First decision: 13 August 2001.

[1] Correspondence. FAX: 81 155 49 5643; hisuzuki@obihiro.ac.jp

[2] Current address: National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, 2-13, Inada, Obihiro, Hokkaido 080-8555, Japan.

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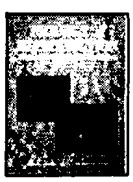

Biology of Reproduction 63, 1294-1302 (2000)

Regular Article

Cryobiology of Rat Embryos I: Determination of Zygote Membrane Permeability Coefficients for Water and Cryoprotectants, Their Activation Energies, and the Development of Improved Cryopreservation Methods[1]

Reinhold T. Pfaff [a,b], **Yuksel Agca** [a,b], **Jun Liu** [a], **Erik J. Woods** [a], **Augustine T. Peter** [b] and **John K. Critser** [2,,a,b]

[a] *Cryobiology Research Institute, Herman B Wells Center for Pediatric Research, Department of Pediatrics, Indiana University School of Medicine, Indianapolis, Indiana 46202 Department of Veterinary Clinical Sciences,* [b] *School of Veterinary Medicine, Purdue University, West Lafayette, Indiana 47907*

ABSTRACT

New rat models are being developed at an exponential rate, making improved methods to cryopreserve rat embryos extremely important. However, cryopreservation of rat embryos has proven to be difficult and expensive. In this study, a series of experiments was performed to characterize the fundamental cryobiology of rat fertilized 1-cell embryos (zygotes) and to investigate the effects of different cryoprotective agents (CPAs) and two different plunging temperatures (T_p) on post-thaw survival of embryos from three genetic backgrounds. In the initial experiments, information on the fundamental cryobiology of rat zygotes was determined, including 1) the hydraulic conductivity in the presence of CPAs (L_p), 2) the cryoprotectant permeability (P_{CPA}), 3) the reflection coefficient (σ), and 4) the activation energies for these parameters. P_{CPA} values were determined for the CPAs, ethylene glycol (EG), dimethyl sulfoxide (DMSO), and propylene glycol (PG). Using this information, a cryopreservation method was developed and the cryosurvival and fetal development of Sprague-Dawley zygotes cryopreserved in either EG, DMSO, or PG and plunged at either -30 or -80°C, were assessed. The highest fetal developmental rates were obtained using a T_p of -30°C and EG (61.2% ± 2.4%), which was not different ($P > 0.05$) from nonfrozen control zygotes (54.6% ± 3.0%).

FOOTNOTES

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[1] Supported by The Cryobiology Research Institute, grant R01-AA120722 from the National Institutes of Health, and Harlan Sprague Dawley, Inc.

[2] Correspondence: John K. Critser, Indiana University School of Medicine, Cancer Research Building, Wells Center for Pediatric Research, 1044 West Walnut St., Room 454, Indianapolis, IN 46202. FAX: 317 274 8679; jcritser@iupui.edu

HUMAN REPRODUCTION

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Human Reproduction, Vol. 14, No. 1, 55-59, January 1999

The mouse as a model to study adhesion formation following endoscopic surgery: a preliminary report

N. Yesildaglar[1,3], J.L. Ordoñez[1], I. Laermans[1] and P.R. Koninckx

[1] Centre for Surgical Technologies (CHT) and [2] Department of Obstetrics and Gynaecology, University Hospital Gasthuisberg, K. U. Leuven, Minderbroederstraat 17, B-3000, Leuven, Belgium

Our aim was to investigate the feasibility of a **mouse model** to study adhesion formation following endoscopic surgery. Following preliminary studies to establish anaesthesia and pneumoperitoneum pressure, a prospective randomized study was carried out to investigate the effect of CO_2 pneumoperitoneum on postoperative adhesions. In group I (control group), the duration of pneumoperitoneum was shorter than 5 min. In groups II, III and IV, pneumoperitoneum was maintained for 60 min without flow, with a continuous low flow (1 ml/min) and a continuous high flow (10 ml/min) through the abdominal cavities of the mice using non-humidified CO_2, respectively. Adhesions were scored after 7 days by laparotomy. The total adhesion scores were 0.9 ± 0.8 (n = 15) in control group, 2.4 ± 0.8 (n = 15) ($P < 0.001$ versus control group) in group II with no flow, 2.6 ± 1.3 (n = 15) ($P < 0.001$ versus control group) in group III with a continuous low flow and 4.3 ± 0.9 (n = 15) ($P < 0.001$ versus control group and $P < 0.001$ versus group II and III) in group IV with a continuous high flow. In conclusion, the mouse can be used as a model to study adhesion formation following endoscopic surgery. Duration of CO_2 pneumoperitoneum is a co-factor in adhesion formation.

Keywords: adhesions • endoscopy • mouse

Presented in part at the International Congress of Peritoneal Tissue Healing, the 4th Peritoneum and Peritoneal Access Meeting, in Göteborg (Gothenburg), Sweden, September 17–19, 1997

[3] To whom correspondence should be addressed

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Hum. Reprod., April 1, 2003; 18(4): 890 - 890.
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Hum. Reprod., June 1, 2002; 17(6): 1623 - 1629.
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Biology of Reproduction 62, 178-185 (2000)

Article

Induction of Apoptosis in Placentas of Pregnant Mice Exposedto Lipopolysaccharides: Possible Involvement of Fas/Fas Ligand System[1]

Kuniaki Ejima [a,b], **Takehiko Koji** [2,c], **Daisuke Tsuruta** [d], **Hiroki Nanri** [a], **Masamichi Kashimura** [b] and **Masaharu Ikeda** [a]

[a] Departments of Health Development and [b] Obstetrics and Gynecology, University of Occupational and Environmental Health, Kitakyushu 807-8555, Japan [c] Department of Histology and Cell Biology, Nagasaki University School of Medicine, Nagasaki 852-8523, Japan [d] Department of Dermatology, Osaka City University Medical School, Osaka 545-8585, Japan

To explore the pathogenesis in placental dysfunction and abruptio placentae, we analyzed the occurrence of placental cell apoptosis and the role of Fas and Fas ligand (L) in that process in an inflammatory placental dysfunction model of pregnant mice, using lipopolysaccharides (LPS). In the present study, Day 13 pregnant mice were injected i.p. with LPS (50 μg/kg) or saline as a control, and the placentas were isolated at various time points after the injection. Analysis of the isolated DNA in agarose-gel electrophoresis revealed a typical ladder pattern of bands consisting of 180–200 base pairs (bp), which is regarded as a hallmark of apoptosis. The intensity of the bands increased time-dependently, reaching a maximum level at 12 h after LPS injection. In accord with the biochemical data, histochemical analysis using terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) revealed that nuclei positive for double-stranded DNA breaks were found in decidua, diploid trophoblasts in the basal zone, and spongiotrophoblasts. The number of positive nuclei was maximized at 12 h after LPS injection. As a next step, we investigated the possible involvement of Fas and Fas L in the induction of apoptosis of the placental cells after LPS injection. Western blot analysis indicated that LPS increased the expression of Fas and Fas L in the placenta by about 4-fold at 12 h and 18 h, respectively, after injection. The cells expressing Fas and Fas L were identified, using immunohistochemistry and nonradioactive in situ hybridization, as decidua, diploid trophoblasts in the basal zone, and spongiotrophoblasts. Furthermore, when the expression of 4-hydroxy-2-nonenal (HNE)-modified proteins was assessed to evaluate the relation of oxidative stress elicited by LPS to the induction of apoptosis, once again decidua, diploid trophoblasts in the basal zone, and spongiotrophoblasts were positive. Therefore, the placental dysfunction by LPS may be brought about by the Fas-mediated apoptosis of various placental cells in a paracrine/autocrine fashion, possibly under the influence of oxidative stress.

First decision: 3 June 1999.

[1] This work was supported by a UOEH Research Grant for promotion of occupational health.

[2] Correspondence: Takehiko Koji, Department of Histology and Cell Biology, Nagasaki University School of Medicine, 1-12-4, Sakamoto, Nagasaki, 852-8523, Japan. FAX: 81 958 497028; tkoji@net.nagasaki-u.ac.jp

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ARTICLES

Diethylstilbestrol-induced cervical and vaginal adenosis using the neonatal mouse model

S Prahalada, VD Castracane, AG Hendrickx and JW Goldzieher
California Primate Research Center, University of California, Davis 95616.

The relevance of diethylstilbestrol (DES) administration to neonatal mice as a model for human pathology attributed to the use of DES in high-risk pregnancies has been investigated, particularly with respect to cervical and vaginal changes in female offspring. Neonatal DES treatment of mice results in tonic pituitary gonadotropin release and continuous estrogen secretion by the ovary. Studies were designed to determine the effect of this altered ovarian endocrine activity on cervical and vaginal histopathology. Ovariectomy of DES-treated mice, with or without estradiol replacement, did not eliminate the lesions, nor did estrogen and progesterone administered in a regimen intended to mimic estrous cycle changes. Induction of the constant estrus state by neonatal estradiol benzoate or testosterone propionate administration or by exposure to constant light did not produce the type of vaginal or cervical changes seen in DES mice. Thus, altered ovarian function is apparently not required for the vaginal and cervical changes appearing in later life. A role for endogenous (or exogenous) ovarian hormones in the developmental progression toward normality is suggested.

Biology of Reproduction, Vol 28, 735-744. Copyright © 1983 by Society for the Study of Reproduction

ARTICLES

Exposure to diethylstilbestrol during pregnancy permanently alters the ovary and oviduct

RR Newbold, BC Bullock and JA Mc Lachlan

To determine the effects of transplacental exposure to diethylstilbestrol (DES) on the ovary and oviduct of the CD-1 mouse, timed pregnant mice were injected subcutaneously with DES (100 micrograms/kg) on Days 9 through 16 of gestation and female offspring sacrificed from 4 weeks to 10 months of age. Following DES exposure, ovarian alterations such as inflammation, a prominent interstitial compartment composed of medullary tubule-like structures, and intra- and para-ovarian cysts from mesonephric remnants were observed. In addition, there were oviductal abnormalities including malformation. As reported previously, the oviduct was closely adherent and coiled around the ovary in a similar position to that seen in the fetal mouse. This malformation was termed developmental arrest of the oviduct (DAO) and was a consistent finding in female offspring exposed prenatally to DES (100 micrograms/kg). Increased prevalence of salpingitis and microscopic alterations in the oviduct were also observed. Oviductal epithelium was mostly secretory type with basal vacuoles. In some cases, oviductal epithelium was hyperplastic and formed mucosal folds resembling glands which extended through the muscularis (diverticulosis). The extent of the adenomatous mucosal folds and the degree of extension through the muscularis increased with the age of the animal (100% at 10 months). Some characteristics of this abnormality resembled salpingitis isthmica nodosa, a lesion described in women which is associated with ectopic pregnancies and subfertility. Gross and microscopic changes in the oviduct were more consistent than were the changes among other portions of the reproductive tract of DES- treated mice previously reported. Since subfertility has been described in this mouse model as well as in prenatally DES-exposed women, the data presented in this report may help in evaluation of the reported reduced fertility in exposed patients as well as other infertility patients.

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Mouse Models for Reproductive Biology Research

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000569 C57BL/6J-*A*$^{w-J}$-*Eda*^{Ta} +/+ *Ar*^{Tfm}

azh
002009 C57BL/6J-*azh*/+

Bax
002994 B6.129X1-*Bax*^{tm1Sjk}

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002612 B6.129S2-*Bmp4*^{tm1Blh}

bs
000385 129S;AKR-*bs*/+

Cga
002494 B6;129S2-*Cga*^{tm1Sac}

Foxp3^{sf}
004088 B.Cg-*Foxp3*^{sf}

Fshb
003283 B6;129S7-*Fshb*^{tm1Zuk}

Gja1
002202 B6.129-*Gja1*^{tm1Kdr}
002201 B6;129S-*Gja1*^{tm1Kdr}

Gnrh^{hpg}
002038 CB17;HPG-*Prkdc*^{scid} *Gnrh*^{hpg}/Bm
000804 HPG/Bm

hop
002718 STOCK *hop*/+ (males only)

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002442 B6.129S4-*Inhbb*^{tm1Jae}
002368 C.129S4(B6)-*Inhbb*^{tm1Jae}

jg
000260 JGBF/Le

jsd (males only)
000708 C57BL/6J-*jsd*/+

Kit^{W} and alleles (germ cell deficient)
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000692 WB/ReJ *Kit*^{W}/+
100410 WBB6F1/J-*Kit*^{W}/*Kit*$^{W-v}$
000350 B6By.Cg-*Kit*$^{W-v}$ *Mitf*$^{Mi-wh}$ *T*
000049 C57BL/6J-*Kit*$^{W-v}$
000194 C57BL/6J-*Lx Kit*$^{W-v}$
100410 WBB6F1/J-*Kit*^{W}/*Kit*$^{W-v}$
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Extracellular sulfatase Sulf-1 precursor (HSulf-1)
gi|33112447|sp|Q8IWU6|SUL1_HUMAN[33112447]

3: P15586 BLink, Domains, Links
N-acetylglucosamine-6-sulfatase precursor (G6S) (Glucosamine-6-sulfatase)
gi|232126|sp|P15586|GL6S_HUMAN[232126]

4: NP_853968 BLink, Domains, Links
PROBABLE SULFATASE [Mycobacterium bovis subsp. bovis AF2122/97]
gi|31791475|ref|NP_853968.1|[31791475]

5: CAD93168 BLink, Domains, Links
PROBABLE SULFATASE [Mycobacterium bovis subsp. bovis AF2122/97]
gi|31617061|emb|CAD93168.1|[31617061]

6: NP_000190 BLink, Domains, Links
N-sulfoglucosamine sulfohydrolase (sulfamidase) [Homo sapiens]
gi|4506919|ref|NP_000190.1|[4506919]

7: NP_055985 BLink, Domains, Links
sulfatase FP [Homo sapiens]
gi|29789064|ref|NP_055985.1|[29789064]

8: NP_061325 BLink, Domains, Links
similar to glucosamine-6-sulfatases; sulfatase 2 [Homo sapiens]
gi|29789100|ref|NP_061325.1|[29789100]

9: NP_000193 BLink, Domains, Links
iduronate-2-sulfatase isoform a precursor [Homo sapiens]
gi|4557659|ref|NP_000193.1|[4557659]

10: NP_002067 BLink, Domains, Links
glucosamine (N-acetyl)-6-sulfatase precursor; N-acetylglucosamine-6-sulfatase; glucosamine-6-sulfatase [Homo sapiens]
gi|4504061|ref|NP_002067.1|[4504061]

11: NP_006114 BLink, Domains, Links
iduronate-2-sulfatase isoform b precursor [Homo sapiens]
gi|5360208|ref|NP_006114.1|[5360208]

12: P22304 BLink Domains Links
Iduronate 2-sulfatase precursor
gi|124174|sp|P22304|IDS_HUMAN[124174]

13: CAA78164 BLink Domains Links
N-acetylglucosamine-6-sulphatase [Homo sapiens]
gi|31867|emb|CAA78164.1|[31867]

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41: AAD45669 BLink, Domains, Links
heparanase [Homo sapiens]
gi|5616197|gb|AAD45669.1|AF152376_1[5616197]

42: AAD45379 BLink, Domains, Links
heparanase [Homo sapiens]
gi|5566374|gb|AAD45379.1|AF165154_1[5566374]

43: AAD41342 BLink, Domains, Links
heparanase [Homo sapiens]
gi|5257454|gb|AAD41342.1|AF144325_1[5257454]